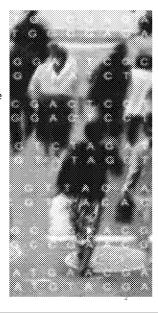


# **NexGen Report Highlights – Executive Summary**

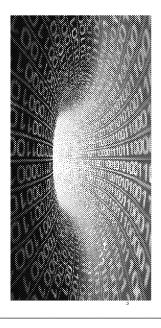
- · Provides the scientific basis for modernizing assessments
  - Engaged a broad array of experts and agencies that conduct risk assessments or risk assessment research
  - Summarized state of the science (>400 references)
  - Created 8 prototype assessments in 3 decision context categories (screening, nonregulatory decision-making and regulatory decision-making)
  - Wrote >40 new NexGen specific technical papers with authorship across the science community
- Illustrates how new science can inform a number of difficult risk assessment issues
  - Causality
  - · Human variability and susceptibility
  - · Cumulative risks
  - · Cross-species relevance
  - · Low exposure-dose-response relationships
- Summarizes challenges and sketches out way forward
  - · Achieved broad agreement among federal agencies and range of experts
  - Identified major challenges for use of new data types
  - · Points to CSS and HHRA research, including new assessment types



# NexGen Overview - Chapter 1

Dramatic progress made towards the vision presented in the NAS Tox21 Century report, and EPA's Future of Toxicity Testing and Risk Assessment report

- Massive amounts of new data and data types are being generated to advance toxicity testing and inform causes of disease
- Most new data types in published literature are now compiled, organized, stored for public use, and sometimes curated
- Automated analytical methods (bioinformatics) to evaluate these large datasets are developing at a rapid pace
- \* Plays to NCEAs strengths in data identification, integration and curation
- NexGen effort engaged the science community and public in a discussion of new approaches to risk assessment
  - · Multiyear, multimillion dollar effort (mostly contributions of others)
  - 6 Federal and state agencies, 3 European agencies, Health Canada, Cal EPA,
     12 universities, Hamner Institute for Health Science and others
  - · External peer and publically reviewed



## **Prelude to Prototypes Development - Chapter 2**

- Identified risk managers' needs, and how new science might address those needs
- 2. Developed:
  - a. Strategy for advancing the next generation of risk assessment (Cote et al. 2011),
  - b. Framework big picture guiding principles for new types of risk assessment (Krewski et al. 2014)
- **3. Presented draft prototypes** to experts for comment and refinement (EPA 2010)
- 4. Initiated public dialogue via workshop and interviews (EPA 2011a) presented prototype concepts; focused on potential applications and communication of progress
- Continued interaction with science community and stakeholders via NAS Emerging Science Workshops and science meetings

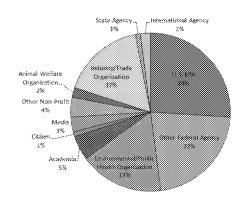


Figure 4. Categories of Stakeholders that Attended the February 2011 NexGen Public Dialogue Conference.

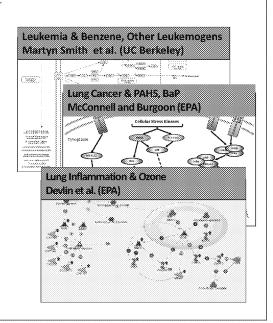
### The Prototypes: 8 Illustrative Examples – Chapter 3

- Tier 3: Major Scope Decision-making: developed proof of concept & explored augmentation of very traditional data-rich chemical assessments
  - · Hematotoxicity and leukemia: benzene & other leukemogens
  - · Lung inflammation and injury: Ozone
  - Lung and liver cancer: benzo[a]pyrene/polycyclic aromatic hydrocarbons
- · Tier 2: Limited Scope Decision-making: explored approaches to assessing hundreds of chemicals
  - Diabetes and obesity: knowledge mining and meta-analyses of published literature
  - Thyroid disruption: short duration in vivo assays alternative species
  - Potency estimates and cancer vs. noncancer: short duration in vivo assays rodent
- · Tier 1: Prioritization and Screening: explored approaches to assessing thousands of chemicals
  - · Potency estimates and some hazard id: quantitative structure activity relationship models
  - Potency estimates and some hazard id: high-throughput and high-content in vitro assays

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### Tier 3 Prototypes: Showed Proof of Concept

- Pattern of "omic" perturbations appear causally linked to human risks for specific diseases/disorders
  - 。 Risk factors disrupt various points in same network
  - Disruptions occur in the same network as naturally occurring disease
  - . Gene variants in the network alter risks
- Biomarkers appear predictive of exposure-response, but to be discerned, must be anchored to traditional data
- Molecular patterns can be used to better characterize:
  - Data limited agents
  - Mixtures & environmental stressors
  - . Human variability in responses
  - Low exposure-dose response
- Variability can be introduced by experimental paradigm, cell & tissue type, lifestage etc.
- Identifies other pollutants that reasonably cause or exacerbate same effects



We next asked about the underlying molecular events and can molecular signatures predict hazards?

We looked at the molecular networks that underly the systems biology model such as shown in the previous slide. This slide continues with the benzene example but the points I will raise are also characteristic of other prototypes in the report.

Shown in this slide is a KEGG (Kyoto Encyclopedia of Genes and Genomics) diagram of the pathways involved in Acute Myeloid Leukemia. This information is drawn from a compilation of many studies, primarily of leukemia of unknown origin, but also of leukemias chemically induced, or modified or ameliorated by therapeutics.

Click... Here I have circled pathways that are altered by benzene exposures at environmental concentrations. One can see that bz disrupts this network at multiple points that would not be well described by a linear AOP.

#### Key Points

Molecular alterations induced by benzene are most closely associated with the pathways involved in leukemia. Leukemias arising de novo, and secondary to benzene and other chemicals exposures appear to share similar mechanisms

Hence, toxicity or adverse outcome pathways are a bit of a misnomer as there are not separate toxic pathways but rather perturbation of normal biology.

Both natural or induced leukemia can occur as a result of perturbations at multiple points of the network, Its not one set of key events that result in leukemia, but more a fuzzy set of events that can occur individually or can be combine in different ways to result in specific diseases.

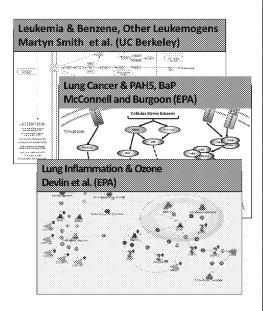
There are also lesion specific differences which comprise distinct cytogenetic subtypes, exhibit different latencies and may arise in cells at different stages of hematopoiesis

To understand how chemicals cause a disease a simple MOA or AOP is not likely to be sufficient.

Characteristic patterns of expression resulting from chemicals exposures appear to predict increased risk of specific outcomes

### Tier 3 Prototypes: Illustrated How IRIS/ISA Assessments Can Be Augmented

- Provides tools for evaluating mechanistic evidence for causality
- Demonstrates omic effects causally linked to disease at US environmental concentrations, supporting low-dose extrapolation
- Illustrates how various chemical and nonchemical risk factors interact
- Provides tools for evaluating extrapolation issues e.g., across species, in vitro to in vivo
- Helps characterize mechanisms for normal & subpopulation sensitivities and related risks
- Develops criteria for evidence evaluation using new data



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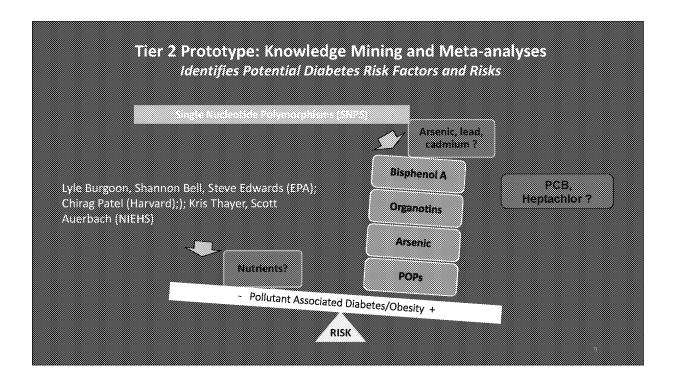
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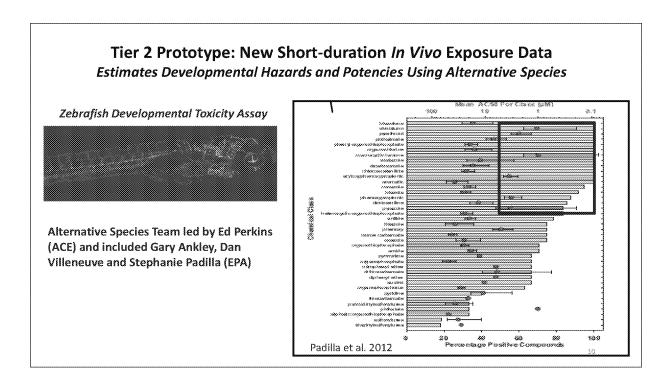
Characteristic patterns of expression resulting from chemicals exposures appear to predict increased risk of specific outcomes



### Tier 2 Prototypes Explored Three General Approaches

- Analyses of existing data using knowledge mining and meta-analyses
- New short-duration in vivo exposure experimental data types
  - Alternative species
  - Rodent species





Percent positive chemicals in each class are represented by the gray bars (bottom axis), and the average AC50 for each group (±SEM) is indicated by the filled red circles (top axis)

Fig. 6. Relationship between chemical class and toxicity to developing zebrafish. 300+ chemicals. The percent positive chemicals in each class are represented by the gray bars (bottom

axis), and the average AC50 for each group (±SEM) is indicated by the filled red circles (top axis). Only classes with three or more total members were analyzed, and only

classes with at least 2 positive chemicals were included in the graph. If a class only had two positive chemicals, no error bars are shown, i.e., triazinylsulfonylurea, aliphatic organothiophosphate, phthalate, thiocarbamate, auxins, diphenyl ether, nitrophenyl ether, and pyrimidine.

# Tier 2 Prototype: New Short-duration In Vivo Exposure Data Estimates Nonspecific PODs Using Lowest Transcriptomic Changes in Rodents

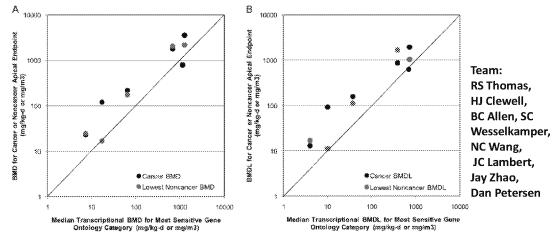
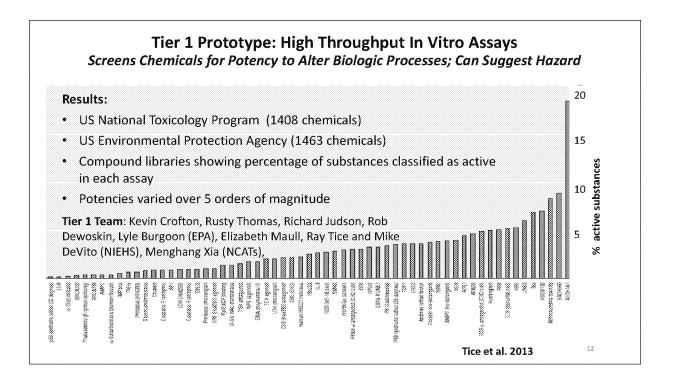


Figure 26. Scatter Plot Correlates Benchmark Values for Cancer and Noncancer Assay Apical Endpoints versus Transcriptional Values for the Most Sensitive Gene Ontology Category. (A) Benchmark Dose; (B) Benchmark Dose Lower Limit. (Thomas et al. 2011).

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1,536-well format

9 human cell types, 2 mouse, 2 rat.

15 concentrations

### Advanced Approaches to Recurring Issues in Risk Assessment - Chapter 4

- Discusses implications of new approaches for recurring issues
- · Recurring issues:
  - Individual vs. Population-level Effects
  - Human Variability and Susceptibility, including Epigenetic and Early Life Exposures
  - Mixtures and Nonchemical Stressors
  - Interspecies Extrapolation
  - Responses at Environmental Exposure Levels

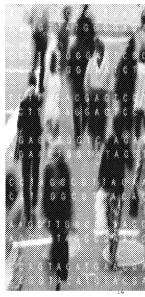


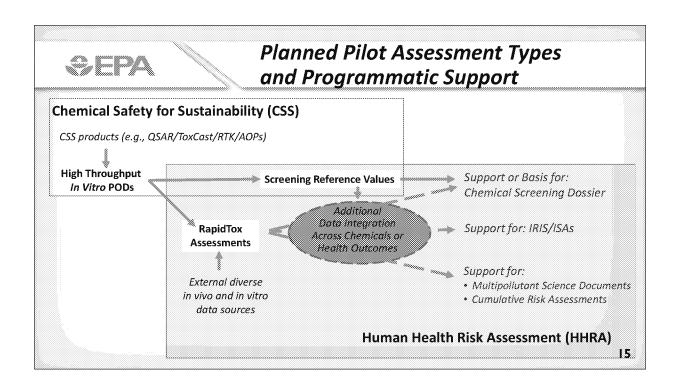
Teams: Lauren Zeise, Frederic Bois, Weihsueh Chiu, Ila Cote, Dale Hattis, Ivan Rusyn, Kathryn Guyton, Lyle Burgoon, Kim Boekelheide, Tim Zarcharewski, Linda Teuschler, Eva McLanahan, Rob Dewoskin, Ed Perkins, Dan Krewski

11.

# **NexGen Program Summary**

- Provides the scientific basis for modernizing assessments –both process and report
- Illustrates how new science can inform a number of difficult risk assessment issues
- · Summarizes challenges and sketches our a way forward
- Program offices are still uncertain about use new approaches and range from wary to enthusiastic about partnering with us to figure it out.





# HHRA/CSS Anticipated Products Schedule:

- 1 year = draft pilot Screening Reference Values
- 2+ years = draft pilot RapidTox assessments on select program office chemicals
- 2-3+ years = support for Air Multipollutant Assessment Document and cumulative risk assessments





#### **Authors**

Managing Editors - Ila Cote, Lyle Burgoon, Rob DeWoskin

#### Authors:

#### **Executive Summary:**

Lyle Burgoon, 1 Rob DeWoskin, 1 Elaine Cohen-Hubal, 1 and Ila Cote1

#### Chapter 1: Introduction

Ila Cote, <sup>1</sup> Paul Anastas, <sup>2</sup> Stan Barone, <sup>1</sup> Linda Birnbaum, <sup>3</sup> Rebecca Clark, <sup>1</sup> Kathleen Deener, <sup>1</sup> David Dix, <sup>1</sup> Stephen Edwards, <sup>1</sup> and Peter Preuss <sup>1</sup>

#### Chapter 2: Preparation for Prototype Development, and Consideration of Decision Context:

- A Framework: Daniel Krewski,<sup>4</sup> Margit Westphal,<sup>4</sup> Greg Paoli,<sup>5</sup> Maxine Croteau,<sup>5</sup> Mustafa Al-Zoughool,<sup>4</sup> Melvin Andersen,<sup>6</sup> Weihsueh Chiu,<sup>1</sup> Lyle Burgoon,<sup>1</sup> and Ila Cote<sup>1</sup>
- Science Community and Stakeholder Engagement: Kim Osborn, 7 Gerald Poje, 8 and Ron White9
- Recurring Issues in Risk Assessment: Daniel Krewski,<sup>4</sup> Melvin Andersen,<sup>6</sup> Kim Boekelheide,<sup>10</sup> Frederic Bois,<sup>11</sup> Lyle Burgoon,<sup>1</sup> Weihsueh Chiu,<sup>1</sup> Michael DeVito,<sup>3</sup> Hisham El-Masri,<sup>1</sup> Lynn Flowers,<sup>1</sup> Michael Goldsmith,<sup>1</sup> Derek Knight,<sup>12</sup> Thomas Knudsen,<sup>1</sup> William Lefew,<sup>1</sup> Greg Paoli,<sup>5</sup> Edward Perkins,<sup>13</sup> Ivan Rusyn,<sup>14</sup> Cecilia Tan,<sup>1</sup> Linda Teuschler,<sup>1</sup> Russell Thomas,<sup>1</sup> Maurice Whelan,<sup>15</sup> Timothy Zacharewski,<sup>16</sup> Lauren Zeise,<sup>17</sup> and Ila Cote<sup>1</sup>

#### **Authors (continued)**

#### Chapter 3; The Prototypes

- Tier 3: Benzene-induced Hematotoxicity and Leukemia: Reuben Thomas,<sup>3</sup> Alan Hubbard,<sup>18</sup> Cliona McHale,<sup>18</sup> Luoping Zhang,<sup>18</sup> Stephen Rappaport,<sup>18</sup> Qing Lan,<sup>19</sup> Nathaniel Rothman,<sup>19</sup> Jennifer Jinot,<sup>1</sup> Babasaheb Sonawane,<sup>1</sup> Martyn Smith,<sup>18</sup> and Kathryn Guyton<sup>1</sup>
- Tier 3: Ozone-induced Lung Inflammation and Injury: Robert Devlin, 1 Kelly Duncan, 1 James Crooks, 1 David Miller, 1 Lyle Burgoon, 1 Michael Schmitt, 1 Stephen Edwards, 1 Shaun McCullough, 1 and David Diaz-Sanchez 1
- Tier 3: Benzo[a]pyrene, Polycyclic Aromatic Hydrocarbons, and Cancer: Lyle Burgoon<sup>1</sup> and Emma McConnell<sup>27</sup>
- Tier 3: Risk Assessment Implications across the Tier 3 Prototypes: Lyle Burgoon, 1 Rob DeWoskin, 1 and Ila Cote1
- Tier 2: Knowledge Mining Diabetes: Lyle Burgoon,<sup>1</sup> Shannon Bell,<sup>27</sup> Chirag Patel,<sup>20</sup> Kristine Thayer,<sup>3</sup> Scott Auerbach,<sup>3</sup> and Stephen Edwards<sup>1</sup>
- Tier 2: Short-term, In Vivo, Alternative Species Data & Endocrine Disruption: Edward Perkins<sup>13</sup>, Gerald Ankley<sup>1</sup>, Stephanie Padilla<sup>1</sup>, Dan Petersen<sup>1</sup>, and Daniel Villeneuve<sup>1</sup>
- Tier 2: Short-term, *In Vivo*, Mammalian Species Data Cancer and Noncance: Michael DeVito,<sup>3</sup> Jason Lambert,<sup>1</sup> Scott Wesselkamper,<sup>1</sup> and Russell Thomas<sup>1</sup>
- Tier 1: Quantitative Structure Activity Relationships and High-throughput Virtual Molecular Docking: Rob DeWoskin,<sup>1</sup> Nina Wang,<sup>1</sup> Jay Zhao,<sup>1</sup> Scott Wesselkamper,<sup>1</sup> Jason Lambert,<sup>1</sup> Dan Petersen,<sup>1</sup> and Lyle Burgoon<sup>1</sup>
- Tier 1: Using High-throughput/High-content Assays: Kevin Crofton, 1 Richard Judson, 1 and Rob DeWoskin 1

:1:3

#### Authors (continued)

#### Chapter 4: Advanced Approaches to Issues in Risk Assessment

- Human Variability including Genomic Variability: Lauren Zeise,<sup>17</sup> Frederic Bois,<sup>11</sup> Weihsueh Chiu,<sup>1</sup> Ila Cote,<sup>1</sup> Dale Hattis,<sup>21</sup> Ivan Rusyn,<sup>14</sup> Kathryn Guyton,<sup>1</sup> and Lyle Burgoon<sup>1</sup>
- Early-life Exposures: Ila Cote<sup>1</sup> and Kim Boekelheide
- Internal Dosimetry: Rob DeWoskin, Ila Cote, and Eva McLanahan
- Mixtures and Nonchemical Stressors: Timothy Zacharewski, <sup>16</sup> Ila Cote, <sup>1</sup> Linda Teuschler, <sup>1</sup> and Lyle Burgoon<sup>1</sup>
- Interspecies Extrapolation: Lyle Burgoon, Illa Cote, and Edward Perkins 13
- Low Dose-Response Characterization: Weihsueh Chiu, Dan Krewski, and Lyle Burgoon 1

#### **Chapter 5: Lessons Learned from Developing the Prototypes:**

Ila Cote, 1 Rob DeWoskin, 1 Lynn Flowers, 1 John Vandenberg, 1 Douglas Crawford-Brown, 22 and Lyle Burgoon 1

#### **Chapter 6: Challenges and Research Directions:**

Rob DeWoskin, Elaine Cohan-Hubal, <sup>1</sup> Tina Bahadori, <sup>1</sup> and Ila Cote<sup>1</sup>

•[¹U.S. Environmental Protection Agency, ²Yale University, ³National Institute of Environmental Health Sciences and the National Toxicology Program, ⁴University of Ottawa, ⁵Risk Sciences International, ⁶The Hamner Institutes for Health Sciences, ¹ICF International, ⁶Grant Consulting Group, ցJohns Hopkins University, ¹¹0Brown University, ¹¹L'Institut National de l'Environnement Industriel et des Risques, ¹²European Chemicals Agency, ¹³Army Corps of Engineers, ¹⁴University of North Carolina at Chapel Hill, ¹⁵European Joint Research Commission, ¹⁶Michigan State University, ¹७California Environmental Protection Agency Office of Environmental Health Hazard Assessment, ¹®University of California at Berkeley, ¹९National Cancer Institute, ²⁰ formerly at Stanford University, now at Harvard University, ²¹Clark University, ²²University of Cambridge, ²³University of Louisville, ²⁴SRC, ²⁵University of Illinois, ²⁶Pfizer, Inc., ²⁵Oak Ridge Institute for Science and Education, ²®International Agency for Research on Cancer]

### **Agency Partners**

- Army Corps of Engineers: Edward Perkins and Anita Meyer
- California Environmental Protection Agency, Office of Environmental Health Hazard Assessment: George Alexeeff, Martha Sandy, and Lauren Zeise
- Centers for Disease Control, National Center for Environmental Health, and the Agency for Toxic
   Substances and Disease Registry: Chris Portier (retired), Bruce Fowler (retired), and Gwendolyn Price
- Department of Defense, Office of the Secretary of Defense: Robert Boyd and Patrick Mason
- · European Chemicals Agency: Derek Knight
- European Joint Research Commission: Maurice Whelan
- FDA's National Center Toxicological Research: Donna Mendrick and William Slikker
- · Health Canada: Carole Yauk
- L'Institut National de l'Environnement Industriel et des Risques: Frederic Bois
- · National Institutes of Health National Center for Advancing Translational Science: Menghang Xia
- National Institute of Environmental Health Sciences and the National Toxicology Program
   Linda Birnbaum, Scott Auerbach, John Balbus, Michael DeVito, Elizabeth Maull, Kristine Thayer, and Ray Tice
- · National Institute for Occupational Safety and Health: Christine Sofge, Paul Schulte, Ainsley Weston

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